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Analysis of PhIP Metabolites by μLC-MS: Development of an Ultra-sensitive Method for Low Dose Detection

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The heterocyclic aromatic amine, PhIP (2-amino-1-methyl-6-phenylimidazo[4,5,b] pyridine), is found in meat products cooked at high temperature and is known to be a potent mutagen and carcinogen in animal models. Here, we describe the development of a highly sensitive micro-liquid chromatography/mass spectrometry (μLC-MS) method for the analysis of metabolic products of PhIP. The method utilizes an on-line sample clean up and pre-concentration procedure followed by LC separation on a micro (1 mm) column, electrospray ionization, and detection via MS or MS/MS. The metabolic products are concentrated on an in-line trap cartridge, washed with an aqueous buffer, and then eluted onto an analytical column. Selective MS/MS detection techniques dramatically reduce much of the background interferences, thus increasing signal-to-noise ratios. Presently, the on-column limit of detection is 10 fmoles (for PhIP) and concentrations down to 500 attomole/μL have been successfully accumulated on the trap cartridge and subsequently detected. The retention efficiency of the metabolites on the trap cartridge ranges from approximately 80% for the most polar to nearly 100% for the least polar. Injection volumes of up to 1 mL have allowed acquisition of metabolic profiles in mouse urine and mothers milk following very low dose exposures (i.e. 100 ng/Kg and 50 ng/Kg respectively). Additionally, analysis at high doses allows the detection of metabolic components not previously detected (e.g. direct glucuronidation metabolites). This paper will report the details of the method and demonstrate the applicability by comparing profiles obtained from various animals and doses.

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